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CLINICAL INVESTIGATION

Plasma insulin-like growth factors and bone formation in uremic hyperparathyroidism

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Plasma insulin-like growth factors and bone formation in uremic hyperparathyroidism. Bone formation in uremia is considered to be regulated in part by parathyroid hormone (PTH). However, while low levels of immunoreactive PTH are usually associated with low rates of bone formation in uremia, elevated PTH levels do not always correlate with increased bone formation. In an attempt to identify other factors that may regulate bone formation in uremic patients, we measured plasma immunoreactive insulin-like growth factors (IGF-I and IGF-II) in 15 patients who did not have aluminum-associated reductions in bone formation. Plasma levels of IGF-I but not PTH, were significantly higher in patients with high rates of bone formation when compared to patients with low or normal bone formation ($P < 0.02$). While the bone formation rate at the tissue level correlated significantly with plasma PTH ($r = 0.53$, $P < 0.05$) and IGF-I ($r = 0.67$, $P < 0.01$), only for plasma IGF-I were there significant correlations with bone apposition ($r = 0.57$, $P < 0.05$) and bone formation rate at the BMU level ($r = 0.62$, $P < 0.02$), parameters which reflect mineralization activity at the cellular level. Among the static histologic parameters, osteoblastic osteoid correlated only with plasma PTH ($r = 0.76$, $P < 0.001$), while osteoclast number correlated with both PTH ($r = 0.56$, $P < 0.05$) and IGF-I ($r = 0.67$, $P < 0.01$). There were no correlations between IGF-II levels and bone histology. From these data we suggest that IGF-I may promote bone formation in uremic patients with hyperparathyroidism. The mechanisms in which IGF-I and PTH enhance bone formation may be different, however, since PTH but not IGF-I correlates with the index for osteoblast number, and IGF-I correlates better with parameters of bone mineralization. IGF-I may directly promote bone matrix mineralization in vivo by stimulating either collagen synthesis or hydroxyapatite formation, or both.

Elevated circulating levels of parathyroid hormone (PTH) are predictive of high bone turnover in uremic patients [1–8]. The anabolic effect of PTH on uremic bone is attributed to enhanced osteoblast activity [3, 8] which is consistent with in vitro studies showing increased proliferation of human osteoblasts following incubation with PTH [9]. Histologically, parameters of bone formation have been shown to correlate directly with PTH levels in several mixed population studies of uremic patients [2–8]. Taken together, these observations have suggested that PTH, when elevated, acts as a major stimulus for bone formation in renal osteodystrophy.

Recently, we demonstrated that plasma amino terminal PTH was directly correlated with bone formation ($r = 0.73$; $P < 0.001$) in a large group of dialysis patients, with and without aluminum-related bone disease [7], consistent with findings from other studies in which amino-terminal PTH assays were also utilized [3, 4]. However, it was also noted that the correlation coefficient between PTH and bone formation was substantially lower ($r = 0.46$) when patients with aluminum-related bone disease were excluded from analysis [7]. Because aluminum is known to suppress both PTH secretion [10, 11] and osteoblast activity [12, 13] in vitro, decreased bone formation in aluminum-related bone disease is likely a consequence of the direct effect of aluminum on bone [14] as well as the indirect effect of lowered PTH levels [15, 16]. Thus, in uremic patients with aluminum bone disease, aluminum and PTH appear to be the major determinants of bone formation, while in those without aluminum osteodystrophy PTH alone accounts for only a portion of the stimulus for bone formation. This suggests to us that, in addition to PTH, other circulating factors may be involved in the regulation of bone formation in uremic hyperparathyroidism.

Insulin-like growth factors (IGFs) are a group of peptides which have growth promoting effects in a variety of cells [17]. In bone organ cultures, Canalis has shown that IGF-I increases DNA and collagen synthesis [18] and Howard and Spencer have shown similar responses to purified IGF-I and IGF-II in chick osteoblast monolayers [19]. More recently, Kurose et al have shown that IGF-I stimulates alkaline phosphatase activity in clonal osteoblasts [20], suggesting a possible role for IGFs in bone mineralization. Because IGFs appear to have an anabolic effect on bone in vitro and since increased IGF levels have been reported in patients undergoing dialysis [21], we reasoned that elevated IGF levels may result in increased bone formation in uremia. Thus, the purpose of this study was to determine whether elevated plasma IGFs are associated with enhanced bone formation in uremic patients without aluminum toxicity and to compare the relationships that the IGFs and PTH may have with parameters of bone formation in patients with secondary hyperparathyroidism.

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Methods

Patients

Previously obtained bone biopsies (double-tetracycline labeled) in 15 patients with chronic renal failure (12 men and 3 women) were selected from a group of 26 patients on the basis of: 1) biopsies having less than 10% stainable bone surface aluminum and 2) the availability of frozen plasma which was obtained within one week of the biopsy. Patients with excess aluminum on bone were excluded because of the suppressive effects of aluminum on bone formation [8, 14] and on PTH secretion [10, 15]. All patients were undergoing thrice weekly hemodialysis either at home or in regional centers. Their mean (±SE) age was 42 ± 3 years and their mean (±SE) duration of dialysis was 65 ± 18 months. None had evidence of liver or thyroid disease, and none had received immunosuppressive therapy prior to the bone biopsy. Six patients were receiving oral calcitriol (mean dose, 0.3 μg/day) and seven patients were receiving calcium carbonate at the time of study. Two patients received both calcitriol and calcium carbonate, and all of the patients were receiving aluminum-containing phosphate binders. There were two patients with diabetes mellitus and two patients who had undergone parathyroidectomy for secondary hyperparathyroidism two and four years before their biopsies. The amino-terminal PTH levels in these patients were 3.5- and 20-fold above the upper normal limit, respectively, at the time of study. All were active and otherwise healthy with no history of recent weight loss.

Biochemistries

Serum calcium, phosphorus, alkaline phosphatase and albumin were determined with an autoanalyzer. Blood for PTH and IGF measurements was obtained before hemodialysis, centrifuged in heparinized tubes and the plasma immediately frozen at −20°C. Immunoreactive PTH was measured in duplicate utilizing an amino-terminal antiserum (CK-67) raised against synthetic human PTH (1-34) [22]. This cross reacts with intact PTH (1-84] but not with mid-region/carboxy-terminal PTH fragments. The normal mean (±SD) is 18 ± 3 pg/ml (N = 61) with a range of 8 to 24 pg/ml. Plasma IGFs were assayed by radioimmunoassay after extraction with acid-ethanol. The method for acid-ethanol extraction by Daughaday, Mariz and Blethen [23] was utilized in which 0.2 ml of plasma sample was treated with 0.8 ml of acidified ethanol for 30 minutes at room temperature. The precipitate was removed by centrifugation and the supernatant neutralized with tris base. The neutralized fraction was then diluted and assayed for IGF-I and IGF-II. The RIA for IGF-I was a procedure described by Copeland, Underwood and Van Wyk [24] in which serum based secondary standard was used routinely. The secondary standard was extracted as above and calibrated against a highly purified IGF-I preparation. Samples were pre-incubated for one hour at 22°C before incubating with ^{125}I-labeled IGF-I (from Nichols Institute Diagnostic) for 18 hours at 4°C. Antibody-^{125}I-IGF-I complexes were then precipitated by goat anti-rabbit gamma globulin precipitating antibody at 4°C and the bound radioactivity in the pellet was counted. The assay has a 2% cross reactivity with IGF-II and <0.01% cross reactivity with proinsulin and pituitary growth hormone. The intra-assay variation is 4.8% and interassay variation is 9.8%. The normal mean (±SD) is 141 ± 21 ng/ml (N = 95) with a range of 51 to 285 ng/ml. The RIA for IGF-II, which uses a monoclonal antibody (from Amano Int. Enzyme Corp., Troy, Virginia, USA), is performed at equilibrium by simultaneous addition of sample and antibody tracer at 4°C for 20 hours. (The ^{125}I-labeled IGF-II was from Drs. Van Wyk and Underwood.) The bound/free separation is achieved using goat anti-mouse gamma globulin. The antibody has 9.6% cross reactivity with IGF-I and <0.01% cross reactivity with proinsulin, insulin, human epidermal growth factor and pituitary growth hormone. The assay is sensitive to 0.2 ng/ml (at 90% B/Bo) using a highly purified standard. The intra-assay variation is 5.3% and interassay variation is 8.6%. The normal mean (±SD) is 606 ± 124 (N = 20) with a range of 358 to 854 ng/ml.

Bone histomorphometry

Histomorphometric analysis of Goldner's stained sections was done as previously described [7]. Static histologic measurements of trabecular bone included osteoid area (as percent of total bone area), osteoid width (μm), osteoblastic osteoid (cuboidal or "plump" osteoblasts) as percent of total bone surface, resorptive surface (as percent of total bone surface), and osteoclast number (expressed as number per mm of bone surface). Normal values for the static parameters used for these comparisons were obtained in our laboratory from 14 male and 6 female subjects (age range, 21 to 59 years). The dynamic measurements were made on unstained trabecular sections and included bone apposition rate (BAR; μm/day) and the length of double-labeled surfaces (Ldl; as percent of total bone surface and as percent of osteoid surface). From these values, the bone formation rate (BFR) at the tissue level was derived by multiplying the bone apposition rate by the double-labeled surfaces, according to the method of Vedi et al [25]. The BFR at the BMU (basic multicellular unit) level was calculated by dividing the BFR at the tissue level by the length of the osteoid surface (expressed as a decimal fraction of the total trabecular surface). Normal values for the dynamic parameters were obtained from the same subjects used in the evaluation of the static parameters.

Statistics

All results are expressed as the mean ±SE unless stated otherwise. The Mann-Whitney rank sum test was used for group comparisons and correlation coefficients were determined using the Spearman rank test for nonparametric regressions with $P < 0.05$ taken as the minimum level of significance. Multiple linear regression analysis was performed as indicated. All computations were performed using the BMDP statistical package (University of California Press, Berkeley, California, USA).

Results

Serum calcium and phosphorus levels were normal or above normal in all patients (Table 1). Serum albumin ranged from 2.9 to 4.5 g/dl with two patients having values below the lower normal limit (2.9 g/dl and 3.4 g/dl, respectively). Plasma PTH levels were markedly elevated in the majority of patients; only two values were in the normal range. Similarly, plasma IGF-I and IGF-II were above the upper normal limit in 47% and 60% of the patients, respectively. There were no significant correla-

Table 1. Biochemical parameters in 15 dialysis patients with hyperparathyroidism

Biochemistries	Patients	Normal
Calcium *mg/dl*	10.4 ± 0.2	8.5–10.5
Phosphorus *mg/dl*	5.9 ± 0.3	2.5–4.5
Albumin *g/dl*	3.9 ± 0.1	3.5–5.5
Alkaline phosphatase *U/liter*	188 ± 38	15–75
PTH *pg/ml*	134 ± 33	18 ± 1[a]
IGF-I *ng/ml*	343 ± 47	141 ± 2[b]
IGF-II *ng/ml*	914 ± 58	606 ± 28[c]

Values are the mean ± SE.
[a] $N = 61$
[b] $N = 95$
[c] $N = 20$

Table 2. Bone histomorphometry in 15 dialysis patients and 20 normal subjects

Histologic parameters	Patients	Normal[a]
Osteoid area *% total bone area*	6 ± 1	4 ± 1
Osteoblastic osteoid *% of total surface*	9 ± 2[b]	3.4 ± 0.6
Osteoclasts *no./mm surface*	3.8 ± 0.9[c]	0.4 ± 0.1
Resorption *% total surface*	17 ± 2[c]	6 ± 1
Fibrosis *% tissue area*	1.7 ± 0.5	0
BAR *μm/day*	0.96 ± 0.08[d]	0.61 ± 0.02
BFR, tissue level *μm³/μm²/day*	0.235 ± 0.125[b]	0.085 ± 0.01
BFR, BMU level *μm³/μm²/day*	0.495 ± 0.082	0.30 ± 0.11
Ldl *% total surface*	22 ± 4	14 ± 1
Ldl *% osteoid surface*	47 ± 6	48 ± 4

Values are the mean ± SE.
[a] Normal values for osteoblastic osteoid and osteoclast number included 13 and 7 subjects, respectively
[b] $P = 0.02$
[c] $P < 0.01$
[d] $P < 0.001$

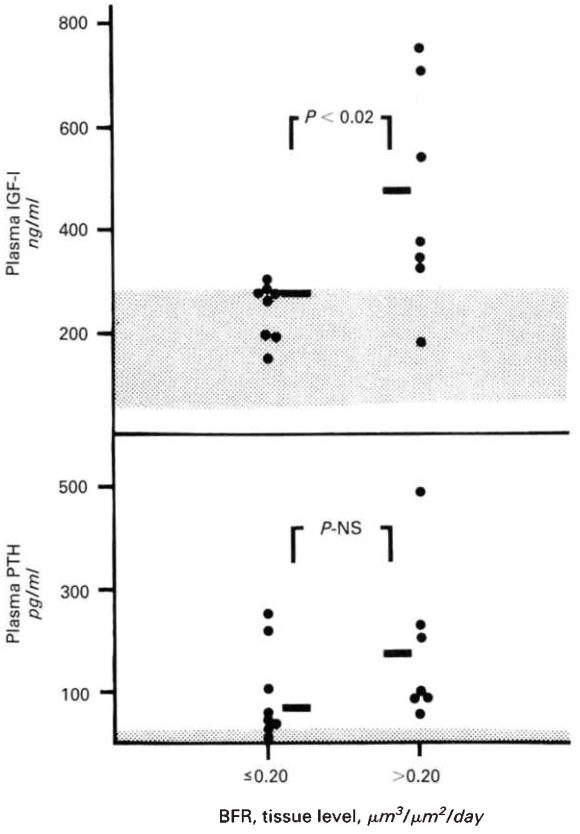

Fig. 1. *Comparisons of plasma IGF-I and PTH in dialysis patients with rates of bone formation below and above 0.20 μm³/μm²/day (upper normal limit).* The shaded areas represent normal ranges.

tions of plasma PTH with IGF-I or with IGF-II and serum albumin did not correlate with either of the plasma IGFs.

To determine whether unsaturated IGF carrier protein was incompletely removed from plasma by the acid-ethanol extraction method, plasma was obtained from five additional dialysis patients and five normal subjects and applied to columns of C_{18} silica (SepPak; Waters Associates, Milford, Massachusetts, USA) as described by Daughaday, Kapadia and Mariz [26]. Briefly, the plasma samples were acidified with 0.5 N HCl and the carrier proteins removed with washes of 4% acetic acid. IGF-I was then eluted with 50% acetonitrile and quantitated by RIA. In the uremic patients the mean (±SD) IGF-I level after acid-ethanol extraction was 187 ± 52 ng/ml and after SepPak extraction, 219 ± 49 ng/ml. In the normal subjects the mean IGF-I level was 125 ± 14 ng/ml after acid-ethanol extraction, and 140 ± 13 ng/ml after SepPak extraction.

All except three of the bone biopsies exhibited histologic values that were either above normal or within the normal range for the parameters evaluated, indicating high-turnover hyperparathyroidism (Table 2). The exceptions were two biopsies which had low values for osteoblastic osteoid (0.5 and 1.2%) and one biopsy which had a low bone formation rate at the tissue level (0.014 $\mu m^3/\mu m^2$/day). This latter biopsy, which was obtained from one of the patients with diabetes, did not show osteomalacia (normal osteoid width and osteoid area). Overall there were seven biopsies with elevated rates of BFR (tissue level). Six of these had a BFR that was greater than five standard deviations above the normal mean (the highest BFR was 11 standard deviations above the normal mean). Marrow fibrosis was moderate in three patients (fibrosis area 0.5 to 2.0% of total tissue area) and severe in six patients (fibrosis area >2.0% of total tissue area).

Displayed in Figure 1 are the comparisons of plasma IGF-I and PTH in relation to the BFR at the tissue level. Among the patients with rates of bone formation greater than the upper normal limit (0.2 $\mu m^3/\mu m^2$/day), all but one had IGF-I levels that exceeded 300 ng/ml whereas none in the group with a normal or low BFR (<0.2 $\mu m^3/\mu m^2$/day) had IGF-I levels above 300 ng/ml. The mean IGF-I levels were significantly different between the two groups (242 ± 19 vs. 459 ± 80 ng/ml, $P < 0.02$). In contrast, the groups with high rates of bone formation did not have PTH levels that were significantly higher than the low BFR group (183 ± 56 vs. 67 ± 28, $P = 0.17$).

In Table 3 are the correlation coefficients for the comparisons of the bone histological parameters and plasma PTH, IGF-I, and IGF-II. Plasma PTH correlated with BFR at the tissue level and the double-labeled surfaces but not with BFR at the BMU level ($r = 0.30$) nor with BAR ($r = 0.23$). In contrast, plasma IGF-I correlated with all the dynamic parameters including BFR at the BMU level ($r = 0.62$, $P < 0.02$) and BAR ($r = 0.57$, $P < 0.05$). Among the static histologic parameters, osteoblastic osteoid was highly correlated with PTH ($r = 0.76$, $P < 0.001$),

Table 3. Correlation coefficients between bone histological parameters and plasma PTH, IGF-I and IGF-II

Parameters	PTH	IGF-I	IGF-II
Dynamic parameters			
BFR, tissue level $\mu m^3/\mu m^2/day$	0.53[d]	0.67[b]	0.33
BFR, BMU level $\mu m^3/\mu m^2/day$	0.30	0.62[c]	0.35
BAR $\mu m/day$	0.23	0.57[d]	0.32
LDl			
% total surface	0.52[d]	0.68[b]	0.34
% osteoid surface	0.53[d]	0.62[c]	0.39
Static parameters			
Osteoblastic osteoid			
% total surface	0.76[a]	0.39	0.13
Osteoid area			
% total bone area	0.35	0.11	−0.05
Osteoclasts *no./mm surface*	0.56[d]	0.67[b]	0.29
Resorption *% total surface*	0.67[b]	0.43	0.48

[a] $P < 0.001$
[b] $P < 0.01$
[c] $P < 0.02$
[d] $P < 0.05$

Table 4. Dynamic bone histologic parameters in dialysis patients with normal and elevated plasma IGF-I

	Plasma IGF-I[a]	
Histologic parameters	Normal $N = 7$	Elevated $N = 8$
BFR tissue level $\mu m^3/\mu m^2/day$	0.128 ± 0.044	0.357 ± 0.062[b]
BFR, BMU level $\mu m^3/\mu m^2/day$	0.269 ± 0.077	0.753 ± 0.067[c]
BAR $\mu m/day$	0.75 ± 0.08	1.20 ± 0.08[c]
Ldl *% total surface*	15 ± 4	31 ± 5[d]
Ldl *% osteoid surface*	33 ± 6	64 ± 5[c]

Values are mean ± SE.
[a] normal range, 51 to 285 ng/ml.
[b] $P = 0.02$ vs. group with normal IGF-I levels
[c] $P < 0.01$ vs. group with normal IGF-I levels
[d] $P = 0.03$ vs. group with normal IGF-I levels

but not with plasma IGF-I ($r = 0.39$). Both plasma PTH and IGF-I correlated with osteoclast number and neither correlated with mineralized bone area. There were no significant correlations between plasma IGF-II and bone histology. Shown in Figures 2 and 3 are the relationships between plasma PTH and IGF-I compared to BFR at the tissue level and to osteoblastic osteoid. Stepwise linear regression analysis using PTH and IGF-I as independent variables revealed no improvement in the prediction of any of the dynamic or static histologic parameters.

As shown in Table 4, all of the dynamic bone parameters listed are significantly higher in the group of patients whose plasma IGF-I levels were elevated above the upper normal limit (>285 pg/ml) compared to those with parameters in the normal range. In addition, the group with elevated IGF-I levels, when compared to normal subjects, also had higher rates of bone formation ($P = 0.02$) as well as higher values for BAR ($P < 0.01$), BFR at the BMU level ($P < 0.01$), Ldl as % of total surface ($P = 0.03$) and Ldl as % of osteoid surface ($P < 0.01$).

Evidence of coupling between bone formation and bone resorption is provided by the positive correlation between osteoblastic osteoid and osteoclast number ($r = 0.83$, $P < 0.0001$) and the correlation between BFR (tissue level) and osteoclast number ($r = 0.78$, $P < 0.001$).

The bone histologic parameters and serum biochemistries were not different between the group of patients that was receiving oral calcitriol ($N = 6$) and the group not taking calcitriol ($N = 9$). In the calcitriol group (mean dose of calcitriol, 0.3 μg/day), the mean (±SD) bone formation rate (tissue level) was 0.14 ± 0.11 $\mu m^3/\mu m^2$/day and the osteoblastic osteoid was 6.8 ± 4.3%. These values are not significantly different from the group not receiving calcitriol: bone formation rate, 0.30 ± 0.20 $\mu m^3/\mu m^2$/day and osteoblastic osteoid, 10.7 ± 8.2%. Moreover, in the calcitriol group, the mean (±SD) serum levels of calcium (10.7 ± 1.0 mg/dl), PTH (121 ± 96 pg/ml) and IGF-I (313 ± 195 ng/ml) are not different from the group not receiving calcitriol: calcium, 10.2 ± 0.7; PTH, 149 ± 147 pg/ml; and IGF-I, 350 ± 188 ng/ml. Finally, there are no differences in bone histology or serum biochemistries between the group of patients receiving calcium carbonate (with or without calcitriol) and the group not receiving calcium carbonate.

Discussion

The finding that plasma IGF-I correlates directly with BFR suggests that this circulating growth factor may be important in promoting bone mineralization in uremia. While correlations of this type do not prove a cause and effect relationship, the finding that patients with elevated plasma IGF-I levels had significantly higher values for all the parameters of bone formation, when compared to those with lower IGF-I levels (Table 4), strengthens the argument for IGF-I having a role in the mineralization process. The exact mechanism in which IGF-I may be anabolic for bone cannot be determined from this study. However, the data suggest that IGF-I may promote bone formation by an effect that is independent of enhanced osteoblast proliferation since IGF-I levels did not correlate with osteoblastic osteoid, an indicator of osteoblast number. Instead, because of the correlations of plasma IGF-I with BAR, Ldl and BFR at the BMU level, IGF-I may be more important in promoting the mineralization of individual bone forming units by either enhancing collagen synthesis or by stimulating the osteoblastic formation of alkaline phosphatase which may be important in the deposition of hydroxyapatite crystals. IGF-I in physiologic concentrations stimulates collagen synthesis in organ bone cultures [18], an effect that is not entirely due to enhanced bone cell proliferation [27]. Moreover, IGF-I increases alkaline phosphatase activity in murine clonal osteoblasts [20]. These data, therefore, strongly suggest that elevated circulating IGF-I may stimulate osteoblast differentiation and thereby directly promote bone mineralization in vivo. The results do not, however, exclude the possibility that the osteoblastic production of IGF-I [28] is also involved in regulating bone formation locally.

Our results are consistent with other reports showing a positive correlation between PTH and various parameters of bone formation in uremia [2–7]. We did not, however, find a significant correlation between PTH and the bone apposition rate as reported elsewhere [7]. The major difference between the current study and earlier reports is our exclusion of patients with aluminum bone disease which was done to eliminate aluminum as a cause of impaired mineralization. Thus, in our study the lack of significant correlations of PTH with BAR and

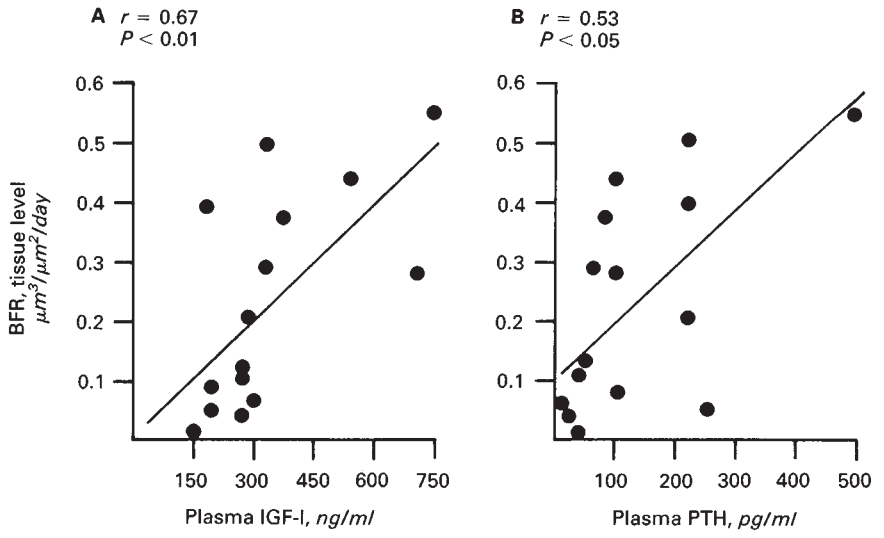

Fig. 2. *Relationship of BFR, tissue level to plasma IGF-I* **(A)** *and to plasma PTH* **(B).**

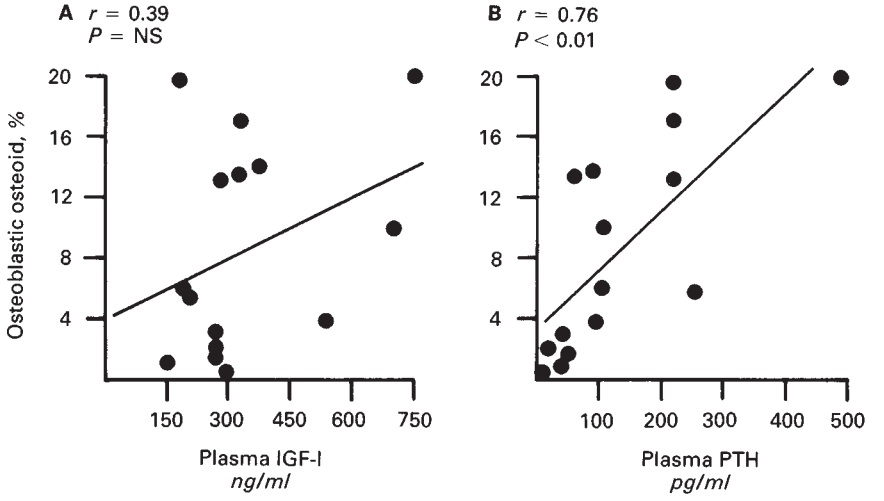

Fig. 3. *Relationship of osteoblastic osteoid to plasma IGF-I* **(A)** *and to plasma PTH* **(B).**

with BFR at the BMU level and the finding that PTH was highly correlated with osteoblastic osteoid suggest that PTH enhances bone formation in uremia primarily by increasing the number of active osteoblasts. Our finding of a positive correlation between PTH and the double-tetracycline labeled surface (Ldl) is attributed to this relationship between PTH and osteoblastic osteoid. Since studies in cultured human bone cell monolayers [9] as well as in rat organ bone cultures [29, 30] have shown that PTH stimulates cell proliferation [9, 30] but decreases collagen synthesis [29], these data would support our suggestion that PTH and IGF-I may have different effects on osteoblast function in vivo.

In addition to the positive correlations between IGF-I and parameters of bone formation, we found that plasma IGF-I also correlated with osteoclast number ($r = 0.67$, $P < 0.01$). While it is possible that IGF-I may stimulate osteoclast activity directly, IGF-I receptors on osteoclasts must first be identified before this can be accepted. A more likely explanation for the correlation is that bone resorption and bone formation have remained coupled in our patients since osteoclast number was highly correlated with osteoblastic osteoid ($r = 0.83$) and BFR ($r = 0.78$). Thus, any stimulus to bone formation, through an effect on the osteoblast, will also increase osteoclast activity and bone resorption. Presently, this is also the explanation for the positive correlation between PTH and osteoclast number, since PTH receptors are lacking in mature osteoclasts [31].

Plasma immunoreactive IGF levels were above the normal range in the majority of our uremic patients. While other studies have reported variable levels of the IGFs in uremic serum [21, 32, 33], none have shown an association between circulating levels and a particular biologic response in this patient population. Because the IGFs circulate bound to large carrier proteins, acid-ethanol pre-treatment of serum samples has been advocated to ensure reliable quantitation of total IGF-I and IGF-II [23]. Recently, it has been proposed that uremic serum contains excess amounts of unsaturated carrier protein which are not completely removed by acid-ethanol extraction, and that underestimation of immunoreactive IGF-I levels can occur as a result of poor separation of antibody and carrier protein by polyethylene glycol [33]. In our IGF radioimmunoassays, a second antibody is used to separate bound from free radioligand, which is more specific than polyethylene glycol in the separation step

[26]. Consequently, unsaturated carrier protein bound to ^{125}I-IGF-I is not precipitated and counted. This explains why the acid-ethanol treated samples from our uremic patients were not lower than normal, in contrast to the results of Powell et al in which polyethylene glycol was used to precipitate the antibody [33]. While acid-ethanol extraction may not completely remove IGF-carrier proteins from uremic plasma, IGF-I levels are underestimated in our RIA by an average of only 17% when compared to IGF-I levels in plasma previously extracted on columns of C_{18} silica to remove carrier protein. Thus, the IGF levels in our patients are an accurate reflection of total IGF-I and are significantly elevated above normal.

The cause of the increased immunoreactive IGF levels in our patients is not apparent. It is possible that IGF production in the liver was stimulated by growth hormone since elevated levels of growth hormone have been observed in uremia [34, 35]. It is also possible that heparinization during dialysis may have contributed to altered IGF levels since heparin is known to prevent binding of IGF-I to its carrier protein [36]. However, we did not find higher IGF-I levels in heparinized uremic plasma when compared to uremic serum from the same patients (data not shown). It is also unlikely that individual differences in the amount of heparin administered during dialysis affected the IGF-I determinations since our patients received similar concentrations of heparin throughout their dialytic treatments and because the plasma measurements were made at least 40 hours after the last heparin infusion.

We do not believe the plasma levels of the IGFs were affected by differences in nutrition. All of our patients were well nourished clinically (only 2 patients had serum albumin levels that were slightly below the lower normal limit) and none had recent weight loss. Furthermore, the serum albumin did not correlate with plasma IGF levels. However, since protein restriction can result in decreased circulating IGF-I [37], this potential situation in a dialysis patient could make IGF-I levels poor predictors of bone formation.

Despite finding a significant relationship between plasma IGF-I and bone histology in this study, we cannot rule out the possibility that circulating IGF inhibitors may also have a role in the bone response to IGF-I. Phillips et al have recently shown that whole uremic serum has decreased IGF activity, as determined by bioassay, primarily as a result of elevated levels of a low molecular weight (<1,000 daltons) inhibitor [38]. Theoretically, this inhibitor could attenuate any anabolic effect that IGF-I may have on bone. This may partially explain why in our study the correlation coefficient between IGF-I and BFR was only 0.67 (R^2 value, 0.45). It remains to be shown, however, whether such inhibitors of IGF action correlate with bone function in uremia, either by inhibiting osteoblast proliferation or by preventing normal bone mineralization.

In summary, we have found that immunoreactive IGF-I levels are directly correlated with rates of bone formation and bone apposition in uremic patients with hyperparathyroidism, suggesting a possible role for IGF-I in promoting bone mineralization. Because both plasma PTH and IGF-I correlate with the bone formation rate at the tissue level but only PTH correlates with the index for osteoblast number, we conclude that PTH and IGF-I may enhance bone formation by different mechanisms. Future studies should investigate the relative contribution of IGF-I in promoting collagen synthesis and hydroxyapatite formation in human bone.

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References

1. Shen FH, Baylink DJ, Sherrard DJ, Shen L, Maloney NA, Wergedal JE: Serum immunoreactive parathyroid hormone and 25-hydroxyvitamin D in patients with uremic bone disease. *J Clin Endocrinol Metab* 40:1009, 1975
2. Teitelbaum SL, Bergefeld MA, Freitag J, Hruska KA, Slatopolsky E: Do parathyroid hormone and 1,25-dihydroxyvitamin D modulate bone formation in uremia? *J Clin Endocrinol Metab* 51:247, 1980
3. Malluche HH, Faugere MC, Fanti P, Price PA: Plasma levels of bone GLA-protein reflect bone formation in patients on chronic maintenance dialysis. *Kidney Int* 26:869, 1984
4. Voigts A, Felsenfeld AJ, Andress DL, Llach F: Parathyroid hormone and bone histology: Response to hypocalcemia in osteitis fibrosa. *Kidney Int* 25:445, 1984
5. Dunstan CR, Hills E, Norman AW, Bishop JE, Mayer E, Wong SYP, Johnson JR, George CRP, Collett P, Kaloski S, Wyndham R, Lawrence JR, Evans RA: The pathogenesis of renal osteodystrophy: role of vitamin D, aluminum, parathyroid hormone and phosphorus. *Quart J Med* 217:127, 1985
6. Chan YL, Furlong TJ, Cornish CJ, Posen S: Dialysis osteodystrophy. *Medicine* 64:296, 1985
7. Andress DL, Endres DB, Maloney NA, Kopp JB, Coburn JW, Sherrard DJ: Comparison of parathyroid hormone assays with bone histomorphometry in renal osteodystrophy. *J Clin Endocrinol Metab* 63:1163, 1986
8. Andress DL, Maloney NA, Endres DB, Sherrard DJ: Aluminum-associated bone disease in chronic renal failure: High prevalence in a long-term dialysis population. *J Bone Miner Res* 1:391, 1986
9. MacDonald BR, Gallagher JA, Russell RGGG: Parathyroid hormone stimulates the proliferation of cells derived from human bone. *Endocrinology* 118:2445, 1986
10. Morrissey J, Rothstein M, Mayor G, Slatopolsky E: Suppression of parathyroid hormone secretion by aluminum. *Kidney Int* 23:699, 1983
11. Bourdeau AM, Plachot JJ, Cournot-Whitmer G, Pointillart A, Balsan S, Sachs C: Parathyroid response to aluminum in vitro: ultrastructural changes in PTH release. *Kidney Int* 331:15, 1987
12. Plachot JJ, Cournot-Whitmer G, Halpern S, Mendez V, Bourdeau A, Fritsch J, Bourdon R, Drueke T, Gaille P, Balsan S: Bone ultrastructure and x-ray microanalysis of aluminum intoxicated hemodialyzed patients. *Kidney Int* 25:796, 1984
13. Lieberherr M, Grosse B, Cournot-Whitmer G, Hermann-Erlee MPM, Balsan S: Aluminum action on mouse bone cell metabolism and response to PTH and 1,25-$(OH)_2D_3$. *Kidney Int* 31:736, 1987
14. Goodman WG, Gilligan J, Horst R: Short-term aluminum administration in the rat: effects on bone formation and relationship to renal osteomalacia. *J Clin Invest* 73:171, 1984
15. Andress DL, Voigts A, Felsenfeld AJ, Llach F: Parathyroid hormone response to hypocalcemia in dialysis patients with osteomalacia and osteitis fibrosa. *Kidney Int* 24:364, 1983

16. GOODMAN WG: Thyroparathyroidectomy modifies the skeletal response to aluminum loading in the rat. *Kidney Int* 31:923, 1987
17. PHILLIPS LS, VASSILOPOULOU-SELLIN R: Somatomedins. *N Engl J Med* 302:371, 1980
18. CANALIS E: Effect of insulin-like growth factor-I on DNA and protein synthesis in cultured rat calvaria. *J Clin Invest* 66:709, 1980
19. HOWARD GA, SPENCER EM: Somatomedins A and C directly stimulate bone cell proliferation in vitro, in *Endocrine Control of Bone and Calcium Metabolism*, edited by COHN DV, FUJITA T, POTTS JT JR, TALMAGE RV, Amsterdam, Excerpta Medica, Vol. 8B, 1984, p 48
20. KUROSE H, SEINO Y, YAMAOKA K, TANAKA H, YABUUCHI H: Effects of somatomedin-C and 1,25dihydroxyvitamin D_3 on clonal osteoblasts, MC3T3 E-1. Abstracts of the Annual Meeting of the American Society for Bone and Mineral Research, June, 1987
21. ENBERG G, HALL K: Immunoreactive IGF-II in serum of healthy subjects and patients with growth hormone disturbances and uremia. *Acta Endocrinol* 107:164, 1984
22. SEGRE GV: Amino-terminal radioimmunoassays for human parathyroid hormone, in *Clinical Disorders of Bone and Mineral Metabolism*, edited by FRAME B, POTTS JT JR, Amsterdam, Excerpta Medica, 1983, p 14
23. DAUGHADAY WH, MARIZ IK, BLETHEN SL: Inhibition of access of somatomedin to membrane receptor and immunobinding sites: Comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. *J Clin Endocrinol Metab* 51:781, 1980
24. COPELAND KC, UNDERWOOD LE, VAN WYK JJ: Induction of immunoreactive somatomedin C in human serum by growth hormone: Dose response relationships and effect on chromatographic profiles. *J Clin Endocrinol Metab* 50:690, 1980
25. VEDI S, COMPSTON JE, WEBB A, TIGHE JR: Histomorphometric analysis of dynamic parameters of trabecular bone formation in the iliac crest of normal British subjects. *Metab Bone Dis Rel Res* 5:69, 1984
26. DAUGHADAY WH, KAPADIA M, MARIZ I: Serum somatomedin binding proteins: Physiologic significance and interference in radioligand assay. *J Lab Clin Med* 109:355, 1987
27. HOCK JM, CENTRELLA M, CANALIS E: Insulin-like growth factor I has independent effects on bone matrix formation and cell replication. *Endocrinology* 122:254, 1988
28. CANALIS E, MCCARTHY T, CENTRELLA M: Isolation and characterization of insulin-like growth factor I (somatomedin-C) from cultures of fetal rat calvariae. *Endocrinology* 122:22, 1988
29. DIETRICH JW, CANALIS EM, MAINA DM, RAISZ LG: Hormonal control of bone collagen synthesis in vitro: Effects of PTH and calcitonin. *Endocrinology* 98:943, 1976
30. DEBARTOLO TF, PEGG LE, SHASSERRE C, HAHN TJ: Comparison of parathyroid hormone and calcium ionopore A23187: Effects on bone resorption and nucleic acid synthesis in cultured fetal rat bone. *Calcif Tiss Int* 34:495, 1982
31. SILVE CM, HRADEK GT, JONES AL, ARNAUD CD: Parathyroid hormone receptor in intact embryonic chicken bone: Characterization and cellular localization. *J Cell Biol* 94:379, 1982
32. GOLDBERG A, TRIVEDI B, DELMEZ J, HARTER H, DAUGHADAY WH: Uremia reduces insulin-like growth factor-I, increases insulin-like growth factor-II and modifies their serum protein binding. *J Clin Endocrinol Metab* 55:1040, 1982
33. POWELL DR, ROSENFELD RG, BAKER BK, LIU F, HINTZ RL: Serum somatomedin levels in adults with chronic renal failure: The importance of measuring insulin-like growth factor-I (IGF-I) and IGF-II in acid-chromatographed uremic serum. *J Clin Endocrinol Metab* 63:1186, 1986
34. SAAMAN N, FREEMAN RM: Growth hormone levels in severe renal failure. *Metabolism* 19:102, 1970
35. DEFRONZO R: Pathogenesis of glucose intolerance in uremia. *Metabolism* 27:1866, 1978
36. CHATELAIN PG, VAN WYK JJ, COPELAND KC, BLETHEN SL, UNDERWOOD LE: Effect of in vitro action of serum proteases or exposure to acid on measurable immunoreactive somatomedin C in serum. *J Clin Endocrinol Metab* 56:376, 1982
37. ISLEY WL, UNDERWOOD LE, CLEMMONS DR: Dietary components that regulate serum somatomedin C concentrations in humans. *J Clin Invest* 71:175, 1983
38. PHILLIPS LS, FUSCO AC, UNTERMAN TG, DEL GRECO F: Somatomedin inhibitor in uremia. *J Clin Endocrinol Metab* 59:764, 1984